FORM PTO-1449 (REV.7-80)	U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE	ATTY. DOCKET NO. 210121.446C2	APPLICATION NO. 09/248,178
INFORMATION DISCLOSURE STATEMENT *(Use several sheets if necessary)*		APPLICANTS Steven G. Reed et al.	
		FILING DATE February 9, 1999	GROUP ART UNIT 1642

U.S. PATENT DOCUMENTS

*EXAMINER INITIAL		DOCUMENT NUMBER	DATE	NAME	CLASS	SUBCLASS	FILING DATE IF APPROPRIATE

FOREIGN PATENT DOCUMENTS

		DOCUMENT NUMBER	DATE	COUNTRY	TRANSLATION YES	TRANSLATION NO

OTHER PRIOR ART *(Including Author, Title, Date, Pertinent Pages, Etc.)*

AA		Genbank Sequence Database, Accession No. N54784, January 28, 1997.
AB		Genbank Sequence Database, Accession No. N59253, February 23, 1996.
AC		Genbank Sequence Database, Accession No. N62351, March 1, 1996.
AD		Genbank Sequence Database, Accession No. N76721, April 2, 1996.
AE		Genbank Sequence Database, Accession No. R75793, June 6, 1995.
AF		Genbank Sequence Database, Accession No. R78938, June 9, 1995.
AG		Genbank Sequence Database, Accession No. T21968, August 5, 1996.
AH		Genbank Sequence Database, Accession No. W02878, April 18, 1996.
AI		Genbank Sequence Database, Accession No. W72837, October 16, 1996.
AJ		Genbank Sequence Database, Accession No. W72838, October 16, 1996.
AK		Genbank Sequence Database, Accession No. Z98046, July 13, 1998.
AL		Liang et al., "Differential Display and Cloning of Messenger RNAS from Human Breast Cancer *Versus* Mammary Epithelial Cell," *Cancer Research 52*:6966-6968, 1992.
AM		Porter Jordan and Lippman, "Overview of the Biological Markers of Breast Cancer," *Breast Cancer 8*:73-100, 1994.
AN		Schlom et al., "Strategies for the Development of Recominant Vaccines for the Immunotherapy of Breast Cancer," *Breast Cancer Research and Treatment 38*(1):27-39, 1996.
AO		Watson and Fleming, "Isolation of Differentially Expressed Sequence Tags from Human Breast Cancer," *Cancer Research 54*(17):4598-4602, 1994.
AP		Yee et al., "Isolation of Tyrosinase-Specific $CD8^+$ and $CD4^+$ T Cell Clones from the Peripheral Blood of Melanoma Patients Following In Vitro Stimulation with Recombinant Vaccinia Virus," *J. of Immunology 157*:4079-4086, 1996.

EXAMINER	DATE CONSIDERED
	10/18/02

* EXAMINER: Initial if reference considered, whether or not criteria is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant(s).

(FILE 'HOME' ENTERED AT 08:13:32 ON 19 OCT 2000)

FILE 'MEDLINE, BIOSIS, CAPLUS, EMBASE, SCISEARCH, BIOTECHDS' ENTERED AT 08:13:36 ON 19 OCT 2000

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L1       315995 S BREAST()(CANCER OR CARCINOMA)
L2         4605 S DETECT?(2W)(BREAST()(CANCER OR CARCINOMA))
L3          969 S DETECT?()(BREAST()(CANCER OR CARCINOMA))
L4           51 S L3 AND (NUCLEOTIDE OR NUCLEIC OR DNA OR CDNA)
L5           11 S L4 AND AMPLIF?
L6            8 S L5 AND PCR
L7            7 DUP REM L6 (1 DUPLICATE REMOVED)
L8          361 S L2 AND (NUCLEOTIDE OR NUCLEIC OR DNA OR CDNA)
L9           75 S L8 AND AMPLIF?
L10          47 S L9 AND PCR
L11          21 DUP REM L10 (26 DUPLICATES REMOVED)

=> d ibib abs 111 1
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L11 ANSWER 1 OF 21 BIOSIS COPYRIGHT 2000 BIOSIS

ACCESSION NUMBER: 2000:207048 BIOSIS
DOCUMENT NUMBER: PREV200000207048
TITLE: Response of circulating tumor cells to systemic therapy in patients with metastatic breast cancer: Comparison of quantitative polymerase chain reaction and immunocytochemical techniques.
AUTHOR(S): Smith, Brendan M.; Slade, Martin J. (1); English, Jacqueline; Graham, Helen; Luchtenborg, Margreet; Sinnett, H. Dudley; Cross, Nicholas C. P.; Coombes, R. Charles
CORPORATE SOURCE: (1) Cancer Research Campaign Laboratories, Division of Cancer Cell Biology, Imperial College School of Medicine, Hammersmith Hospital, Du Cane Rd, London, W12 ONN UK
SOURCE: Journal of Clinical Oncology, (April, 2000) Vol. 18, No. 7,
pp. 1432-1439.
ISSN: 0732-183X.
DOCUMENT TYPE: Article
LANGUAGE: English
SUMMARY LANGUAGE: English

AB Purpose: We previously developed a quantitative system for the detection of cytokeratin 19 (CK-19) transcripts using reverse transcriptase polymerase chain reaction (**PCR**) to **detect** **breast carcinoma** cells in blood and bone marrow. The aim of this study was to determine the value of this system in monitoring patients with metastatic disease and to compare it with an established immunocytochemical method. Patients and Methods: Patients with progressive, locally advanced, and metastatic breast cancer (all stage IV) who were due to start systemic treatment were recruited. Blood samples were analyzed for CK-19 transcripts using quantitative **PCR** (QPCR) and immunocytochemistry (ICC) throughout their course of treatment. Results: One hundred forty-five blood samples were obtained from 22 patients over 13 months. Seventy-two (49.6%) of these samples were positive by QPCR, and 56 (42%) of 133 were positive by ICC. Of the 133 specimens analyzed by both techniques, 95 (71.4%) had the same results for each, and of the 71 samples that were positive, 40 (56%) were positive by both methods. The relationship between the number of cells detected and the QPCR values was statistically significant (P < .0001). Of the 25

courses of assessable treatment, 17 (68%) of 25 treatment outcomes (either response or disease progression) were reflected by QPCR measurements, and 12 (57%) of 21 were reflected by ICC. During the course of the study, five patients showed a response, and of these, ICC was in agreement in four cases (80%) and QPCR in three cases (60%). Eighteen courses of treatment resulted in progression of the disease; however, only 15 of these were assessable by ICC. ICC was in agreement in eight (53%) of 15 of these cases, and QPCR in 15 (83%) of 18 cases. Conclusion: Circulating carcinoma cells are frequently found in patients with metastatic breast cancer. In the majority of patients, cancer cell numbers as evaluated by QPCR or ICC reflected the outcome of systemic treatment.

=> d ibib abs l11 2

L11 ANSWER 2 OF 21 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1999:468628 CAPLUS
DOCUMENT NUMBER: 131:101263
TITLE: Human mammary-associated chemokine MACK and its use for detection and treatment of breast disease
INVENTOR(S): Papsidero, Lawrence D.; Dyster, Lyn M.; Frustaci, Jana M.
PATENT ASSIGNEE(S): Codon Diagnostics, LLC, USA
SOURCE: PCT Int. Appl., 76 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9936540	A1	19990722	WO 1999-US651	19990112
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 9921134	A1	19990802	AU 1999-21134	19990112
PRIORITY APPLN. INFO.:			US 1998-71899	19980120
			US 1998-92155	19980709
			WO 1999-US651	19990112

AB An isolated human mammary-assocd.chemokine (MACK) is disclosed. Suppression subtractive hybridization showed that the isolated chemokine is expressed preferentially in breast tissue or can be detected in breast milk, and can be **detected** in **breast cancer** sera using Western blotting. MACK includes from about 100 to about 132 amino acids, has a deduced mol. wt. of from about 10 to about 16 kDa, and has a deduced isoionic point of from about pH 10.1 to about pH 10.7. Antibodies and binding portions thereof recognizing the subject chemokine and peptides which include the antigenic portions of the subject chemokines are described. **DNA** mols. which encode the subject chemokines as well as **nucleic** acid mols. which, under stringent conditions, hybridize to **nucleic** acid mols. encoding the subject chemokines or to a complement thereof are also disclosed. The chemokines, peptides, antibodies, and binding portions thereof, and **nucleic** acid mols. can be used to detect and treat breast disease, such as

inflammations, infections, mastitis, benign cystitis, benign hyperplasias,
cancer and other malignancies as well as other pathol. states of the mammary gland.
REFERENCE COUNT: 7
REFERENCE(S): (1) Anon; Database EMBL 1995
(2) Anon; Database EMBL 1995
(3) Dumas, M; WO 9906549 A 1999
(4) Edwards, J; WO 9906439 A 1999
(6) Srivastava, M; RESEARCH COMMUNICATIONS IN MOLECULAR PATHOLOGY AND PHARMACOLOGY 1996, V93(3),
P263 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d ibib abs l11 3

L11 ANSWER 3 OF 21 MEDLINE
ACCESSION NUMBER: 1999146287 MEDLINE
DOCUMENT NUMBER: 99146287
TITLE: p73 at chromosome 1p36.3 is lost in advanced stage neuroblastoma but its mutation is infrequent.
AUTHOR: Ichimiya S; Nimura Y; Kageyama H; Takada N; Sunahara M; Shishikura T; Nakamura Y; Sakiyama S; Seki N; Ohira M; Kaneko Y; McKeon F; Caput D; Nakagawara A
CORPORATE SOURCE: Division of Biochemistry, Chiba Cancer Center Research Institute, Japan.
SOURCE: ONCOGENE, (1999 Jan 28) 18 (4) 1061-6.
Journal code: ONC. ISSN: 0950-9232.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199904
ENTRY WEEK: 19990404
AB p73, a novel p53 family member, is a recently identified candidate neuroblastoma (NBL) suppressor gene mapped at chromosome 1p36.33 and was found to inhibit growth and induce apoptosis in cell lines. To test the hypothesis that p73 is a NBL suppressor gene, we analysed the p73 gene in primary human NBLs. Loss of heterozygosity (LOH) for p73 was observed in 19% (28/151) of informative cases which included 92 mass-screening (MS) tumors. The high frequency of p73 LOH was significantly associated with sporadic NBLs (9% vs 34%, P<0.001), N-myc **amplification** (10% vs 71%, P<0.001), and advanced stage (14% vs 28%, P<0.05). Both p73alpha and p73beta transcripts were detectable in only 46 of 134 (34%) NBLs at low levels by RT-**PCR** methods, while they were easily **detectable** in most **breast cancers** and colorectal cancers under the same conditions. They found no correlation between p73 LOH and its expression levels (P>0.1). We found two mutations out of 140 NBLs, one somatic and one germline, which result in amino acid substitutions in the C-terminal region of p73 which may affect transactivation functions, though, in the same tumor samples, no mutation of the p53 gene was observed as reported previously. These results suggest that allelic loss of the p73 gene may be a later event in NBL tumorigenesis. However, p73 is infrequently mutated in primary NBLs and may hardly function as a tumor suppressor in a classic Knudson's manner.

=> d ibib abs l11 4

L11 ANSWER 4 OF 21 EMBASE COPYRIGHT 2000 ELSEVIER SCI. B.V.
ACCESSION NUMBER: 1999348018 EMBASE

TITLE: Unreliability of carcinoembryonic antigen (CEA) reverse transcriptase-polymerase chain reaction (RT-**PCR**) **detecting** contaminating **breast cancer** cells in peripheral blood stem cells due to induction of CEA by growth factors.

AUTHOR: Goeminne J.-C.; Guillaume T.; Salmon M.; Machiels J.-P.; D'Hondt V.; Symann M.

CORPORATE SOURCE: Dr. J.-C. Goeminne, Lab. Experimental Oncology Hematol., Catholic University of Louvain, Avenue Hippocrate 54, UCL 54, 71 1200 Brussels, Belgium

SOURCE: Bone Marrow Transplantation, (1999) 24/7 (769-775). Refs: 51
ISSN: 0268-3369 CODEN: BMTRE

COUNTRY: United Kingdom

DOCUMENT TYPE: Journal; Article

FILE SEGMENT: 016 Cancer
022 Human Genetics
025 Hematology
037 Drug Literature Index

LANGUAGE: English

SUMMARY LANGUAGE: English

AB RT-**PCR** is increasingly used for the detection of minimal residual disease in solid tumors. Carcinoembryonic antigen (CEA) RT-**PCR** seemed to be highly specific for detection of tumor cells when tested on PBMC. A very high frequency of RT-**PCR** **amplification** product for CEA in PBSC from breast cancer patients mobilized with G-CSF was found. However, this result contrasted with tumor cell detection by immunocytochemistry (ICC) which showed no correlation with RT-**PCR** results. In addition, CEA mRNA was **amplified** in most G-CSF-mobilized PBSC samples derived from patients with hematological malignancies and from healthy donors of allogeneic stem cells, although no circulating epithelial cells could be demonstrated by ICC. CEA RT-**PCR** expression was observed in PBMC from healthy individuals incubated in vitro with G-CSF. These data suggest that CEA transcription can be induced by GCSF, resulting in a loss of specificity of CEA RT-**PCR** for tumor cell detection in PBMC. We conclude, CEA RT-**PCR** may not be recommended to detect tumor cell contamination in peripheral blood from patients treated with G-CSF. This may have implications on tumor cell detection by RT-**PCR** in tissues where endogenous or exogenous growth factors may induce the transcription of CEA or other genes.

=> d ibib abs l11 5

L11 ANSWER 5 OF 21 CAPLUS COPYRIGHT 2000 ACS

ACCESSION NUMBER: 1999:253239 CAPLUS

DOCUMENT NUMBER: 131:125993

TITLE: Reliable and sensitive detection of premature termination mutations using a protein truncation test designed to overcome problems of nonsense-mediated mRNA instability

AUTHOR(S): Bateman, John F.; Freddi, Susanna; Lamande, Shireen R.; Byers, Peter; Nasioulas, Steven; Douglas, Jenny; Otway, Robyn; Kohonen-Corish, Maija; Edkins, Edward; Forrest, Susan

CORPORATE SOURCE: Orthopaedic Molecular Biology Research Unit, Department of Paediatrics, Royal Children's Hospital, University of Melbourne, Parkville, 3052, Australia

SOURCE: Hum. Mutat. (1999), 13(4), 311-317
CODEN: HUMUE3; ISSN: 1059-7794

PUBLISHER: Wiley-Liss, Inc.

DOCUMENT TYPE: Journal

LANGUAGE: English

AB The protein truncation test (PTT) is a mutation-detection method used to scan for premat[redacted] termination (nonsense) mutati[redacted]. **PCR amplification** of the **DNA** or mRNA source material is performed using forward primers contg. a T7-promoter sequence and translation initiation signals such that the resultant products can be transcribed and translated in vitro to identify the smaller truncated protein products. MRNA is commonly used as the source material, but success of the PTT and other RNA-based mutation detection methods can be severely compromised by nonsense mutation-induced mRNA decay, a well-documented process that is often overlooked in mutation detection strategies. In this study, we develop an RNA-based PTT that overcomes the problem of mRNA decay by preincubating cells with cycloheximide to stabilize the mutant mRNA. The effectiveness of this method for mutation detection in abundant mRNAs was demonstrated in osteogenesis imperfecta fibroblasts by the protection of type I collagen (COL1A1) mRNA contg. nonsense mutations that normally resulted in mutant mRNA degrdn. Stabilization of mutant mismatch repair gene (MLH1) mRNA was also obsd. in transformed lymphocytes from patients with hereditary nonpolyposis colorectal cancer (HNPCC). Importantly, our strategy also stabilized very low-level (or illegitimate) nonsense-contg. transcripts in lymphoblasts from patients with Bethlem myopathy (COL6A1), familial adenomatous polyposis (APC), and breast cancer (BRCA1). The greatly increased sensitivity and reliability of this RT-**PCR**/PTT protocol has broad applicability to the many genetic diseases in which only blood-derived cells may be readily available for anal.

REFERENCE COUNT: 27

REFERENCE(S):
(1) Andreutti-Zaugg, C; Cancer Res 1997, V57, P3288 CAPLUS
(2) Bala, S; Hum Genet 1996, V98, P528 CAPLUS
(3) Bateman, J; Biochem J 1984, V217, P103 CAPLUS
(5) Bernard, M; Biochemistry 1983, V22, P5213 CAPLUS
(6) Carter, M; EMBO J 1996, V15, P5965 CAPLUS
ALL CITATIONS AVAILABLE IN THE RE FORMAT

=> d ibib abs l11 6

L11 ANSWER 6 OF 21 MEDLINE DUPLICATE 1

ACCESSION NUMBER: 2000037737 MEDLINE
DOCUMENT NUMBER: 20037737
TITLE: Comparison of immunocytochemistry, reverse transcriptase polymerase chain reaction, and **nucleic** acid sequence-based **amplification** for the **detection** of circulating **breast cancer** cells.
AUTHOR: Lambrechts A C; Bosma A J; Klaver S G; Top B; Perebolte L; van't Veer L J; Rodenhuis S
CORPORATE SOURCE: Division of Experimental Therapy, The Netherlands Cancer Institute, Amsterdam.
SOURCE: BREAST CANCER RESEARCH AND TREATMENT, (1999 Aug) 56 (3) 219-31.
Journal code: A8X. ISSN: 0167-6806.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200003
ENTRY WEEK: 20000304

AB Detection of tumor cells in blood and bone marrow is increasingly used for the staging of patients with breast cancer and to evaluate the presence of

tumor cells in peripheral blood progenitor cell collections to be used after high-dose therapy. We evaluated the sensitivity and specificity of three different methods for detection of tumor cells among non-tumor tissue. An immunocytochemical assay using antibodies directed against epitopes of the cytokeratin-19 (CK19) protein and two RNA-based methods: reverse transcriptase polymerase chain reaction (RT-**PCR**) and **Nucleic** Acid Sequence-Based **Amplification** (NASBA) for the same target gene were tested. With all the three methods, false-positive results were observed when peripheral blood mononuclear cells (PBMC) of healthy volunteers were tested. There was no concordance between the RNA-based assays and the immunocytochemical assay. The false-positive results in the RNA-based assays may be due to 'illegitimate expression' of epithelial genes in normal PBMC. The false-positive results in the immunocytochemical assay resulted from background staining of monocytes and granulocytes. This study demonstrates that CK19 is not a suitable target to detect the presence of breast tumor tells in PBMC. To reliably detect circulating tumor cells with RNA methods, the selection of suitable target genes is required, which are highly expressed in tumors but not at all in normal cells of blood and bone marrow. Genes with such characteristics may be identifiable with novel differential display techniques.

=> d ibib abs l11 7-21

L11 ANSWER 7 OF 21 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 2000:203474 CAPLUS
DOCUMENT NUMBER: 133:203477
TITLE: Quantitation of erbB-2 gene copy number in breast cancer by an improved polymerase chain reaction (**PCR**) technique, competitively differential **PCR**
AUTHOR(S): Deng, Guoren; Kim, Young S.
CORPORATE SOURCE: Department of Medicine, University of California and VA Medical Center, San Francisco, CA, USA
SOURCE: Breast Cancer Res. Treat. (1999), 58(3), 213-217
CODEN: BCTRD6; ISSN: 0167-6806
PUBLISHER: Kluwer Academic Publishers
DOCUMENT TYPE: Journal
LANGUAGE: English
AB A new method of measuring gene copy no. in small samples of **DNA** was used to measure **amplification** of the erbB-2 gene and a ref. gene in breast cancers. The method, termed 'competitively differential polymerase chain reaction' (CD-**PCR**), combines the advantages of two other techniques for measuring **amplification** by **PCR**, namely differential **PCR** (D-**PCR**) and competitive **PCR** (C-**PCR**). The CD-**PCR** methodol. was evaluated for sensitivity and specificity by comparing **amplification** measured by CD-**PCR** with that obtained by fluorescence in situ hybridization (FISH), C-**PCR**, and Southern blotting anal. CD-**PCR** anal. proved to be an accurate predictor of **amplification**. CD-**PCR** also overcomes the problems involved in variation of **PCR** efficiencies and **DNA** concns. in tumor samples, and the problems caused by the plateau effect in **PCR**.
REFERENCE COUNT: 6
REFERENCE(S): (1) Deng, G; Breast Cancer Res and Treat 1996, V40, P271 CAPLUS
(2) Deng, G; Nucleic Acids Res 1993, V21, P4848 CAPLUS
(3) Frye, R; Oncogene 1989, V4, P1153 CAPLUS

(4) Higuchi, R; Biotech 1993, V11, P1026 MEDLINE
(5) Kallioniemi, O; Proc Natl Acad Sci USA 1992, V89, P5321 CAPLUS
ALL CITATIONS AVAILABLE IN T[redacted]RE FORMAT

L11 ANSWER 8 OF 21 CAPLUS COPYRIGHT 2000 ACS
ACCESSION NUMBER: 1998:543170 CAPLUS
DOCUMENT NUMBER: 129:171517
TITLE: Breast cancer specific gene 1
INVENTOR(S): Ji, Hongjun; Rosen, Craig A.
PATENT ASSIGNEE(S): Human Genome Sciences, Inc., USA
SOURCE: PCT Int. Appl., 73 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9833915	A1	19980806	WO 1998-US1804	19980203
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
AU 9862572	A1	19980825	AU 1998-62572	19980203
EP 1015582	A1	20000705	EP 1998-904776	19980203
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
PRIORITY APPLN. INFO.:			US 1997-37080	19970203
			WO 1998-US1804	19980203

AB **CDNA** sequences are provided encoding the human Breast cancer specific gene 1 [BCSG1] protein. BCSG1 polypeptides are also provided as are vectors, host cells and recombinant methods for producing the same. Lastly, diagnostic methods for **detecting breast cancer** are described.

L11 ANSWER 9 OF 21 MEDLINE
ACCESSION NUMBER: 1998239426 MEDLINE
DOCUMENT NUMBER: 98239426
TITLE: Putative markers for the **detection** of **breast carcinoma** cells in blood.
AUTHOR: Eltahir E M; Mallinson D S; Birnie G D; Hagan C; George W D; Purushotham A D
CORPORATE SOURCE: University Department of Surgery, Western Infirmary, Glasgow, UK.
SOURCE: BRITISH JOURNAL OF CANCER, (1998 Apr) 77 (8) 1203-7. Journal code: AV4. ISSN: 0007-0920.
PUB. COUNTRY: SCOTLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199807
ENTRY WEEK: 19980705

AB The aim of this study was to investigate certain genes for their suitability as molecular markers for **detection** of **breast carcinoma** cells using the reverse transcriptase-polymerase chain reaction (RT-**PCR**). RNA was prepared from MCF-7 breast carcinoma cells and peripheral blood leucocytes of healthy female volunteers. This RNA was screened for mRNA of MUC1, cytokeratin 19 (CK19) and CD44 (exons 8-11) by RT-**PCR** and the results validated by Southern blots. Variable degrees of expression of MUC1 and CD44 (exons 8-11) were detected

in normal peripheral blood, rendering these genes non-specific for epithelial cells and therefore unsuitable for use as markers to **detect breast carcinoma** cells. Although CK19 mRNA was apparently specific, it was deemed unsuitable for use as a marker of breast cancer cells in light of its limited sensitivity. Furthermore, an attempt at using nested primers to increase sensitivity resulted in CK19 mRNA being detected after two **amplification** rounds in blood from healthy volunteers.

L11 ANSWER 10 OF 21 MEDLINE DUPLICATE 2
ACCESSION NUMBER: 1999047091 MEDLINE
DOCUMENT NUMBER: 99047091
TITLE: Cyclin D1 gene **amplification** and protein expression in benign breast disease and breast carcinoma.
AUTHOR: Zhu X L; Hartwick W; Rohan T; Kandel R
CORPORATE SOURCE: Department of Pathology and Laboratory Medicine, Mount Sinai Hospital, University of Toronto, Ontario, Canada.
SOURCE: MODERN PATHOLOGY, (1998 Nov) 11 (11) 1082-8.
Journal code: PTH. ISSN: 0893-3952.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199903
ENTRY WEEK: 19990301
AB Cyclin D1 plays a critical role in regulating cell-cycle progression. Gene **amplification** and protein overexpression of cyclin D1 have been **detected** in **breast cancer** but little is known concerning whether these changes occur in normal breast tissue and in breast lesions associated with increased risk of development of invasive breast cancer. We looked for cyclin D1 gene **amplification** and protein overexpression in 30 cases of benign breast disease (16 epithelial hyperplasias without atypia and 14 atypical ductal hyperplasias) and 18 ductal carcinomas in situ by use of differential **PCR** and immunohistochemical staining. We compared the resulting frequencies to those in 15 cases of normal breast tissue and 17 invasive ductal carcinomas. We found cyclin D1 gene **amplification** in 15% of those with normal breast tissue, 19% of those with epithelial hyperplasia without atypia, 27% of those with atypical ductal hyperplasia, 35% of those with ductal carcinoma in situ, and 25% of those with invasive ductal carcinoma; corresponding figures for protein overexpression were 13, 13, 57, 50, and 64%. These results suggest that cyclin D1 **amplification** and protein overexpression can occur before histologic alterations are seen but that the frequencies of these changes are higher in histologic lesions with cellular atypia (atypical hyperplasia and ductal carcinoma in situ), reaching frequencies similar to those observed in invasive carcinoma.

L11 ANSWER 11 OF 21 MEDLINE DUPLICATE 3
ACCESSION NUMBER: 1998400113 MEDLINE
DOCUMENT NUMBER: 98400113
TITLE: Human immunodeficiency virus type 1-like **DNA** sequences and immunoreactive viral particles with unique association with breast cancer.
AUTHOR: Rakowicz-Szulczynska E M; Jackson B; Szulczynska A M; Smith M
CORPORATE SOURCE: Departments of Obstetrics and Gynecology, University of Nebraska Medical Center, Omaha, Nebraska, USA.. EMRAKOWI@UNMC.edu
SOURCE: CLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY, (1998 Sep) 5

(5) 645-53.
Journal code: CB7. ISSN: 1071-412X.
PUB. COUNTRY: [redacted]ted States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
OTHER SOURCE: GENBANK-AF073463; GENBANK-AF073464; GENBANK-AF073465; GENBANK-AF073466; GENBANK-AF073467; GENBANK-AF073468; GENBANK-AF073469
ENTRY MONTH: 199812
ENTRY WEEK: 19981204

AB RAK antigens p120, p42, and p25 exhibit molecular and immunological similarity to the proteins encoded by human immunodeficiency virus type 1 (HIV-1) and are expressed by 95% of breast and gynecological cancer cases in women and prostate cancer cases in men. The binding of an epitope-specific anti-HIV-1 gp120 monoclonal antibody (MAb) (amino acids 308 to 322) to cancer RAK antigens has been found to be inhibited by a peptide derived from variable loop V3 of HIV-1. Breast cancer **DNAs** of 40 patients were **PCR amplified** with HIV-1 gp41-derived primers, and all of the samples were found to be positive. The **DNA** fragments **amplified** in seven blindly selected breast cancer samples were sequenced. The breast cancer **DNA** sequences showed at least 90% homology to the HIV-1 gene for gp41. Antisense oligonucleotides complementary to the HIV-1-like sequences inhibited reverse transcriptase activity and inhibited the growth of breast cancer cells in vitro. Viral particles **detected** in **breast cancer** cell lines were strongly immunogold labeled with the anti-HIV-1 gp120 MAb. The results obtained strongly suggest that the long-postulated breast cancer virus may, in fact, be related to HIV-1.

L11 ANSWER 12 OF 21 SCISEARCH COPYRIGHT 2000 ISI (R)
ACCESSION NUMBER: 1998:513715 SCISEARCH
THE GENUINE ARTICLE: ZX117
TITLE: **Detection** of occult **breast cancer** micrometastases in axillary lymph nodes using a multimarker reverse transcriptase-polymerase chain reaction panel
AUTHOR: Lockett M A; Baron P L; OBrien P H; Elliott B M; Robison J G; Maitre N; Metcalf J S; Cole D J (Reprint)
CORPORATE SOURCE: MED UNIV S CAROLINA, DEPT SURG, ROOM 420 CSB, 171 ASHLEY AVE, CHARLESTON, SC 29425 (Reprint); MED UNIV S CAROLINA, DEPT SURG, CHARLESTON, SC 29425; HOLLINGS CANC CTR, DEPT SURG, CHARLESTON, SC; HOLLINGS CANC CTR, DEPT PATHOL, CHARLESTON, SC
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF THE AMERICAN COLLEGE OF SURGEONS, (JUL 1998) Vol. 187, No. 1, pp. 9-16.
Publisher: AMER COLL SURGEONS, 54 EAST ERIE ST, CHICAGO, IL 60611.
ISSN: 1072-7515.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE; CLIN
LANGUAGE: English
REFERENCE COUNT: 29

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Background: Axillary lymph node status in breast cancer patients remains the single most important predictor of outcomes. Current methods of histopathologic analysis may be inadequate because 30% of node-negative patients recur. The purpose of this study was to test the hypothesis that a multigene reverse transcriptase-polymerase chain reaction (RT-**PCR**) panel provides a more sensitive method to detect axillary lymph node metastases than routine pathologic examination.

Study Design: Sixty-one consecutive breast cancer patients were evaluated, with nine normal control patients. Nodes > 1 cm were bisected for histopathol[redacted]c and RT-**PCR** analysis. Nodal t[redacted]ue was homogenized, and total RNA was converted into **cDN**[redacted] with reverse transcriptase. Reverse transcriptase-polymerase chain reaction analysis was performed with primers specific for keratin-19, c-myc, prolactin inducible protein (PIP), and beta-actin using ethidium bromide gel electrophoresis. Reverse transcriptase-polymerase chain reaction positive/
pathology negative axillary lymph nodes were reevaluated using step sectioning and immunohistochemical staining.

Results: Thirty-seven patients had pathologically negative axillary lymph nodes, of which 15 (40%) were positive by RT-**PCR** analysis. Two RT-**PCR** negative results tone probably from tissue processing error and the other secondary to sampling error) among the 24 histologically positive specimens were detected (8%). The number of patients in each pathologic stage was 26 patients in stage I; 18, stage IIA; 7, stage IIB; 7, stage IIIA; 3, stage IIIB; and 0 patients in stage TV. By RT-**PCR** staging, 8 of 26 patients went from stage I to IIA (30%), and 7 of 18 from stage IIA to IIB (39%). Of the RT-**PCR** positive individuals who were stage I by pathologic analysis, 100% were found to be c-myc positive, 0% keratin-19 positive, and 0% PIP positive; for stage IIIB patients these markers were 50%, 100%, and 100% respectively. Additionally, an increasing number of positive markers per specimen appeared to correlate with larger primary tumor size ($p < 0.01$) and decreased predicted 5-year survival ($r = 0.950$, $p < 0.002$). Conclusions: Multimarker RT-**PCR** analysis appears to be a readily available and highly sensitive method for the detection of axillary lymph node micrometastases. Longterm followup of RT-**PCR** positive patients will be required to determine its clinical relevance. If validated as a predictor of disease recurrence, this method would provide a powerful complement to routine histopathologic analysis of axillary lymph nodes. (J Am Coil Surg 1998;187:9-16. (C) 1998 by the American College of Surgeons).

L11 ANSWER 13 OF 21 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 4
ACCESSION NUMBER: 1997:238415 CAPLUS
DOCUMENT NUMBER: 126:221461
TITLE: **Detection** of circulating **breast cancer** cells by RT-**PCR** **amplification** of their prostate-specific antigen mRNA or other specific components
INVENTOR(S): Lehrer, Steven
PATENT ASSIGNEE(S): Lehrer, Steven, USA
SOURCE: PCT Int. Appl., 27 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9707242	A1	19970227	WO 1996-US10647	19960620

W: JP, US
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

PRIORITY APPLN. INFO.:	US 1995-2468	19950816
	US 1995-561952	19951122

AB An effective method for the **detection** of circulating **breast cancer** cells is provided. Based on the observation that one third of breast cancer cells produce prostate-specific antigen (PSA), a clin. test employs a reverse transcriptase (RT)-polymerase chain reaction (**PCR**) assay utilizing oligonucleotide primers specific for **nucleic** acids encoding PSA. The assay identifies PSA-synthesizing cells from reverse

transcribed mRNA. Breast carcinoma cell lines can be induced to produce PSA by treatment with androgens, progestins, mineralocorticoids, glucocorticoids or antiestrogens. The assay is applied to RNAs extd. from the peripheral blood of breast cancer patients, and can recognize one PSA-expressing metastatic breast cancer cell dild. into 105 white blood cells. Other **amplification** methods are also provided to detect PSA mRNA or, alternatively, **nucleic** acids encoding other antigens expressed by breast cancer cells.

L11 ANSWER 14 OF 21 MEDLINE DUPLICATE 5
ACCESSION NUMBER: 97390897 MEDLINE
DOCUMENT NUMBER: 97390897
TITLE: Minimal illegitimate levels of cytokeratin K19 expression in mononucleated blood cells detected by a reverse transcription **PCR** method (RT-**PCR**).
AUTHOR: Lopez-Guerrero J A; Bolufer-Gilabert P; Sanz-Alonso M; Barragan-Gonzalez E; Palau-Perez J; De la Rubia-Comos J; Sempere-Talens A; Bonanad-Boix S
CORPORATE SOURCE: Department of Clinical Biochemistry, Hospital La Fe, Valencia, Spain.
SOURCE: CLINICA CHIMICA ACTA, (1997 Jul 4) 263 (1) 105-16. Journal code: DCC. ISSN: 0009-8981.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199712
AB Cytokeratin K19 (CK19) expression was evaluated by a reverse transcription **PCR** method (RT-**PCR**) in the RNA obtained from peripheral blood stem cell collections (PBSC) from four patients with breast cancers (BC) and 34 mononucleated blood cell (MBC) negative controls (17 PBMC from normal subjects 12 PBSC from different types of leukaemias--M3, M4Eo, M2, etc.--and two from patients with Hodgkin's lymphoma; and three bone marrow (BM) collections). Two BC tissues were taken as positive controls. The method studied (Datta YH, Paul T, Adams PT, Drobyski WR. Sensitive **detection** of occult **breast cancer** by reverse transcription polymerase chain reaction. J Oncol 1994;12:475-8) is sensitive enough to allow the detection of CK19 transcripts in a 10(-6) dilution of **cDNA** reverse transcribed from 1 microgram of BC RNA, but CK19 transcripts were also detected in 64% of the RNA obtained from the MBC controls. However, the **amplified** product detected in the control samples represents the transcript of the CK19 gene as confirmed by the results of Mae III digestion. It should be pointed out that although the CK19 expression was detected, the levels of expression in PBMC were almost negligible for they disappeared at 1:5 **cDNA** dilution. Moreover, a direct relationship between the number of BC cells added to PBMC and the increasing dilution levels of the **cDNA** necessary to prevent CK19 expression was observed. This allows us to conclude that the **cDNA** dilutions make it possible to distinguish the false from the true positive samples and that, in addition, the **cDNA** dilutions inform about the degree of BC cell contamination.

L11 ANSWER 15 OF 21 MEDLINE
ACCESSION NUMBER: 96163299 MEDLINE
DOCUMENT NUMBER: 96163299
TITLE: **Detection** of **breast cancer** micrometastases in axillary lymph nodes by means of reverse transcriptase-polymerase chain reaction. Comparison between MUC1 mRNA and keratin 19 mRNA **amplification**.

AUTHOR: Noguchi S; Aihara T; Motomura K; Inaji H; Imaoka S; Koyama H

CORPORATE SOURCE: [redacted]partment of Surgery, Center fo[redacted]dult Diseases, Osaka, Japan.

SOURCE: AMERICAN JOURNAL OF PATHOLOGY, (1996 Feb) 148 (2) 649-56.

Journal code: 3RS. ISSN: 0002-9440.

PUB. COUNTRY: United States

Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals; Cancer Journals

ENTRY MONTH: 199605

AB Usefulness of MUC1 mRNA and keratin 19 mRNA as a target of reverse-transcriptase polymerase chain reaction (RT-**PCR**) was compared in the **detection** of **breast cancer** micrometastases in axillary lymph nodes. RT-**PCR** **amplification** of MUC1 mRNA and keratin 19 mRNA was conducted using total RNA samples. RT-**PCR** products were stained with ethidium bromide and analyzed by agarose gel electrophoresis. Expression of both MUC1 mRNA and keratin 19 mRNA was detected by RT-**PCR** in a breast cancer cell line (MRK) and in all the 23 primary breast cancers but not in the control lymph nodes obtained from patients with benign diseases. A serial dilution study of MRK cells against normal lymph node cells has shown that detection sensitivity of MUC1 RT-**PCR** and keratin 19 RT-**PCR** were 1/10(5) and 1/10(6) (cancer/lymph node cells), respectively. Sixty-three axillary lymph nodes were obtained from 23 patients with primary breast cancer, and metastases in each lymph node were investigated by histological examination (hematoxylin and eosin sections) and RT-**PCR** method. In all 10 lymph nodes, which were histologically metastasis-positive, both MUC1 mRNA and keratin mRNA were detected by RT-**PCR**. Of the 53 histologically negative lymph nodes, 3 (6%) and 5 (9%) lymph nodes were found to express MUC1 mRNA and keratin 19 mRNA, respectively, indicating the presence of micrometastases which could be detected by RT-**PCR** but not by histological examination. These results demonstrate the usefulness of both MUC1 RT-**PCR** and keratin 19 RT-**PCR** in the **detection** of **breast cancer** micrometastases in lymph nodes, and also indicate the superiority of keratin 19 RT-**PCR** over MUC1 RT-**PCR** because of its higher detection sensitivity.

L11 ANSWER 16 OF 21 MEDLINE DUPLICATE 6

ACCESSION NUMBER: 97213564 MEDLINE

DOCUMENT NUMBER: 97213564

TITLE: [Levels of cytokeratin CK19 expression in mononuclear blood cells evaluated using a reverse **PCR** (RT-**PCR**)].
Niveles de expresion de la citoqueratina CK19 en celulas sanguineas mononucleadas evaluada mediante un metodo de **PCR** reversa (RT-**PCR**).

AUTHOR: Lopez Guerrero J A; Bolufer P; Barragan E; Sanz Alonso M; Palau J; Sempere A; De la Rubia J; Bonanad S; Torregrosa M D

CORPORATE SOURCE: Departmento de Biopatologia Clinica, Hospital Universitario La Fe, Valencia.

SOURCE: SANGRE, (1996 Dec) 41 (6) 441-6.
Journal code: U93. ISSN: 0036-4355.

PUB. COUNTRY: Spain

Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: Spanish

ENTRY MONTH: 199707

AB AIMS: The sensitivity and specificity of a reverse transcription **PCR** method (RT-**PCR**) to detect cytokeratin K19 (CK19)

expression was evaluated with the purpose of assessing its capability to detect the presence of breast cancer tumour cells in peripheral blood progenitor cell collection that had to be reinfused to breast cancer patients submitted to intensive chemotherapy as hematopoietic support. PATIENTS AND METHODS: Two breast cancers as positive samples were used and 34 samples of mononucleated blood cells as negative controls: 18 peripheral blood samples from normal subjects, 14 from different types of leukaemias (M3, M4Eo, M2, etc.) and two from two patients with Hodgkin's lymphoma. The method studied is a nested RT-**PCR** that **amplifies** the CK19 expression from the sample RNA extracted following the method of phenol-chloroform. RESULTS: The right performance of the method is demonstrated by observing the detection of CK19 transcripts in the breast cancer RNA and by obtaining good blank results both with non transcribed RNA and with **DNA**. Moreover, the method has an excellent sensitivity such as to allow the detection of CK19 transcripts in a 10(-6) dilution of **cDNA** reverse transcribed from 1 microgram of breast cancer RNA. The CK19 transcripts were also detected in the 64% of RNA obtained from the mononucleated blood cells controls, although the percentage of positivities was lower (47%) in the RNA from peripheral blood samples. Nevertheless it should be remarked that the levels of CK19 expression in the blood mononucleated cells is almost negligible since it used to extinguish at 1:5 **cDNA** dilution. CONCLUSIONS: The method studied is specific and has a high sensitivity that explains the detection of CK19 illegitimate expression approximately a half in mononucleated blood cells negative controls. However, the levels of CK19 expression in mononucleated blood cells were almost negligible and it used to extinguish at 1:5 **cDNA** dilution, therefore it could be concluded that the method might be useful to **detect** **breast cancer** occult tumours cells in mononucleated blood cell collection, always provided that a lower amount of **cDNA** is taken, thus decreasing to nil almost the false positive samples and keeping always a good sensitivity.

L11 ANSWER 17 OF 21 MEDLINE DUPLICATE 7
ACCESSION NUMBER: 96331347 MEDLINE
DOCUMENT NUMBER: 96331347
TITLE: **Detection** of **breast cancer** micrometastases in lymph nodes by **amplification** of keratin 19 mRNA with reverse-transcriptase polymerase chain reaction.
AUTHOR: Noguchi S; Aihara T; Motomura K; Inaji H; Koyama H
CORPORATE SOURCE: Dept. of Surgery, Center for Adult Diseases, Osaka, Japan.
SOURCE: GAN TO KAGAKU RYOHO [JAPANESE JOURNAL OF CANCER AND CHEMOTHERAPY], (1996 Mar) 23 Suppl 1 50-5. Ref: 9
Journal code: 6T8. ISSN: 0385-0684.
PUB. COUNTRY: Japan
Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LANGUAGE: Japanese
FILE SEGMENT: Cancer Journals; Priority Journals
ENTRY MONTH: 199611

AB Keratin 19 mRNA reverse-transcriptase polymerase chain reaction (K 19 RT-**PCR**) was compared with histological examination in the **detection** of **breast cancer** micrometastases in axillary lymph nodes. Sixty-three axillary lymph nodes, which were obtained from 23 breast cancer patients, were bisected. One half was studied by conventional histological analysis of hematoxylin and eosin sections. Total RNA was extracted from the other half and subjected to K 19 RT-**PCR**. In all the ten lymph nodes, which were histologically metastasis-positive, K 19 mRNA was detected by RT-**PCR**. Of the 53

histologically negative lymph nodes, five (9%) lymph nodes were found to express K 19 mRNA, indicating the presence of micrometastases which could be detected by [redacted]**PCR** but not by a histological [redacted]mination. These results de[redacted]nstrate the usefulness of K 19 [redacted]**-PCR** in the **detection** of **breast cancer** micrometastases in lymph nodes.

L11 ANSWER 18 OF 21 CAPLUS COPYRIGHT 2000 ACS DUPLICATE 8
ACCESSION NUMBER: 1994:526225 CAPLUS
DOCUMENT NUMBER: 121:126225
TITLE: **Detection** of **breast cancer** micrometastases in Axillary Lymph Nodes by Using Polymerase Chain Reaction
AUTHOR(S): Schoenfeld, A.; Luqmani, Y.; Smith, D.; O'Reilly, S.; Shousha, S.; Sinnett, H. D.; Coombes, R. C.
CORPORATE SOURCE: Dep. Surg., Charing Cross Hosp., London, UK
SOURCE: Cancer Res. (1994), 54(11), 2986-90
CODEN: CNREA8; ISSN: 0008-5472
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Breast cancer micrometastases in axillary lymph nodes have been detected by serial sectioning and immunohistochem., and shown to have prognostic significance. The authors have used polymerase chain reaction (**PCR**) to see whether the authors could further improve the detection rate of micrometastases. Fifty-seven axillary lymph nodes from patients with breast cancer were examd. histol. to assess the proportion of tumor involvement. Immunohistochem. staining with the use of an anti-keratin 19 antibody confirmed the histol. findings. Reverse transcription **PCR** was then performed on extd. RNA by using K19 primers, and all 18 histol. involved nodes yielded the expected 460-base pair product. Of 39 histol. neg. nodes, 4 (10%) gave K19 bands detectable with ethidium staining and a further 10 (28%) gave K19 bands after Southern hybridization. To further increase the detection sensitivity a two stage **amplification** was performed by using nested primers, and K19 product was found in lymph nodes from patients without cancer, as well as in all the nodes from cancer patients. This was shown to be genuine low level expression from endogenous mRNA template, and not derived from **amplification** of a K19 pseudogene. Reducing the no. of **PCR** cycles in the two **amplification** steps did not allow sufficient discrimination between normal nodes and those involved nodes in which K19 expression was only detectable after Southern hybridization. The optimal "cut-off" point to distinguish involved nodes from normal nodes remained at the level of 40 cycles of **PCR** and Southern hybridization. **PCR**, using K19 as a tumor marker, has been demonstrated in this study to improve the detection of micrometastases in axillary lymph nodes in patients with breast cancer: sensitivity is limited by the specificity of the tumor marker.

L11 ANSWER 19 OF 21 MEDLINE DUPLICATE 9
ACCESSION NUMBER: 94340509 MEDLINE
DOCUMENT NUMBER: 94340509
TITLE: The **detection** of **breast carcinoma** micrometastases in axillary lymph nodes by means of reverse transcriptase-polymerase chain reaction.
AUTHOR: Noguchi S; Aihara T; Nakamori S; Motomura K; Inaji H; Imaoka S; Koyama H
CORPORATE SOURCE: Department of Surgery, Center for Adult Diseases, Osaka, Japan.
SOURCE: CANCER, (1994 Sep 1) 74 (5) 1595-600.
Journal code: CLZ. ISSN: 0008-543X.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Abridged Index Medicus Journals; Priority Journals; Cancer

Journals
ENTRY MONTH: 199411
AB BACKGROUND. The development of a sensitive method for the **detection** of **breast carcinoma** micrometastases in axillary lymph nodes is reported. METHODS. The method was based on **amplification** of MUC1 mRNA, which encodes a core protein of polymorphic epithelial mucin, by a reverse transcriptase-polymerase chain reaction (RT-**PCR**). Total RNA, which was extracted from a breast carcinoma cell line (MCF-7), primary breast carcinomas, and axillary lymph nodes, was subjected to analysis of MUC1 mRNA expression by the RT-**PCR** method. RESULTS. MUC1 mRNA expression was detected by RT-**PCR** in MCF-7 cells and in all 15 primary breast carcinomas but not in control lymph nodes taken from patients with benign diseases. A serial dilution study revealed that MUC1 RT-**PCR** was a very sensitive method, detecting one MCF-7 cell per 1,000,000 lymph node cells. The detection sensitivity of MUC1 RT-**PCR** method was compared with that of immunohistochemical staining of an epithelial marker (polymorphic epithelial mucin). Fifty axillary lymph nodes were obtained from 15 patients with primary breast carcinomas, and metastasis in each lymph node was investigated by both methods. The immunohistochemical method demonstrated metastasis in nine lymph nodes, and MUC1 mRNA was detected in all of them. Of the 41 lymph nodes that were diagnosed to be devoid of metastasis by immunohistochemistry, MUC1 mRNA was expressed by 6 but not by the other 35, indicating the presence of micrometastases in these 6 lymph nodes that could be detected only by the MUC1 RT-**PCR** method. CONCLUSIONS. The MUC1 RT-**PCR** method is more sensitive than immunohistochemistry for the detection of micrometastases in axillary lymph nodes. This new method would be of practical value in selecting the patients at high risk for relapse from those who are histologically lymph node negative.

L11 ANSWER 20 OF 21 MEDLINE DUPLICATE 10
ACCESSION NUMBER: 96265172 MEDLINE
DOCUMENT NUMBER: 96265172
TITLE: **Detection** of **breast cancer** -associated estrone sulfatase in breast cancer biopsies and cell lines using polymerase chain reaction.
AUTHOR: Evans T R; Rowlands M G; Luqmani Y A; Chander S K; Coombes R C
CORPORATE SOURCE: Department of Medical Oncology, St George's Hospital Medical School, London, England.
SOURCE: JOURNAL OF STEROID BIOCHEMISTRY AND MOLECULAR BIOLOGY, (1993 Aug) 46 (2) 195-201.
Journal code: AX4. ISSN: 0960-0760.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals; Cancer Journals
ENTRY MONTH: 199610
AB Steroid sulfatase (STS) is a single enzyme with a range of substrate specificities, including estrone sulfate. Using a 2.4 kb **cDNA** clone, expression of human STS was undetectable by Northern hybridization, but STS RNA was detected in human placenta, human breast cancer samples, and in breast carcinoma cell lines following reverse transcriptase-**PCR amplification**, using specific primers to yield a product of 472 bp. In preliminary studies, stimulation of MCF-7 cell lines with estradiol (10(-8) M) resulted in an increased level of **amplifiable** STS RNA, and this upregulation of STS RNA could be abolished by tamoxifen. The estrone sulfatase activity in mammary tumors

derived from N-nitrosomethylurea (NMU) treated rats was significantly decreased in animals treated with tamoxifen compared to control animals, regardless of the response of the tumors to the [redacted]iestrogen ($P < 0.05$). Although tamoxifen does not inhibit the estrone sulfatase enzyme in vitro, it may modulate the expression of STS RNA and the enzyme activity in vivo.

L11 ANSWER 21 OF 21 MEDLINE DUPLICATE 11
ACCESSION NUMBER: 94122030 MEDLINE
DOCUMENT NUMBER: 94122030
TITLE: The **detection** of a **breast cancer** antigen on MCF-7 cells reactive with the TCR (alpha) of a specific killer T cell line.
AUTHOR: Okano F; Tachibana H; Akiyama K; Murakami H
CORPORATE SOURCE: Graduate School of Genetic Resources Technology, Kyushu University, Fukuoka, Japan.
SOURCE: BIOTHERAPY, (1993) 6 (3) 195-203.
Journal code: AU3. ISSN: 0921-299X.
PUB. COUNTRY: Netherlands
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199405

AB We established several immortalized human T cell lines by cotransfecting primary lymphocytes derived from breast cancer patients with human c-myc and human c-Ha-ras oncogenes. A CD8 positive (CD8+) killer T cell line, FT-8, exhibited in vitro specific cytotoxicity to a human breast cancer cell line, MCF-7. The FT-8 cells suppressed the growth of MCF-7 cells transplanted to athymic mice. The cytotoxic reaction was caused via T cell antigen receptor (TCR) on MCF-7 cells, because monoclonal antibodies against the TCR inhibited the cytotoxicity of FT-8 cells. The TCR (alpha) **cDNA** of FT-8 was cloned by using a **PCR amplification** technique and expressed by a cell-free in vitro translation system. The TCR (alpha) protein recognized a target antigen of 32 KDa on MCF-7 cells.